Effect of twice-yearly denosumab on prevention of bone mineral density loss in de novo kidney transplant recipients: a randomized controlled trial

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Effect of twice-yearly denosumab on prevention of bone mineral density loss in *de novo* kidney transplant recipients: a randomized controlled trial

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Abbreviations: aBMD and vBMD, areal and volumetric bone mineral density; BSAP, bone-specific alkaline phosphatase; β-CTX, C-terminal telopeptide of type I collagen; DXA, dual-energy x-ray absorptiometry; HR-pQCT, high-resolution peripheral quantitative computer tomography; P1NP, procollagen type I N-terminal propeptide; RANKL, receptor activator of nuclear factor κB ligand

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Abstract

We conducted an open-label, prospective, randomized trial to assess the efficacy and safety of RANKL inhibition with denosumab to prevent the loss of BMD in the first year after kidney transplantation. Ninety kidney transplant recipients were randomized 1:1 two weeks after surgery to receive denosumab (60 mg at baseline and 6 months) or no treatment. After 12 months, total lumbar spine aBMD increased by 4.6% (95% CI 3.3-5.9%) in 46 patients in the denosumab group and decreased by -0.5% (95% CI -1.8-0.9%) in 44 patients in the control group (between-group difference 5.1% (95% CI 3.1-7.0%), *P*<0.0001). Denosumab also increased aBMD at the total hip by 1.9% (95% CI, 0.1 to 3.7%; *P*=0.035) over that in the control group at 12 months. HR-pQCT in a subgroup of 24 patients showed that denosumab increased vBMD at the distal tibia and radius (all *P*<0.05). Biomarkers of bone turnover (β-CTX, P1NP) markedly decreased with denosumab (all *P*<0.0001). Episodes of cystitis and asymptomatic hypocalcemia occurred more often with denosumab, whereas graft function, rate of rejections and incidence of opportunistic infections were similar. In conclusion, denosumab increased BMD in the first year after kidney transplantation but was associated with more frequent episodes of urinary tract infection.

Introduction

Patients with progressing chronic kidney disease develop significant alterations in bone and mineral metabolism, including renal osteodystrophy, osteomalacia, adynamic bone disease and decreased bone mineralization (1). Kidney transplant candidates often present with osteopenia or osteoporosis which aggravate further following kidney transplantation, particularly within the first 6 to 12 months after engrafting (2). This leads to a 3-fold increase in the risk for fractures and contributes to long-term morbidity after kidney transplantation and reduced quality of life (3).

The post-transplant loss of bone mass is mainly caused by the immunosuppressive treatment, particularly corticosteroids which often cannot be avoided, and by persisting hyperparathyroidism which causes phosphate wasting by the functioning graft (4). Therapeutic options to limit bone loss include generous supplementation with calcium and vitamin D (5, 6), but their use is limited in transplant recipients with persistent hyperparathyroidism and hypercalcemia. Although bisphosphonates improve bone loss (7-10), their use has not become widespread, particularly because of concerns regarding their nephrotoxicity, and because of controversial data on their efficacy in preventing fractures in renal transplant recipients (11, 12). Furthermore, bisphosphonates have been associated with the occurrence of atypical fractures in this patient population (13, 14). Thus, the management of bone loss and osteoporosis after kidney transplantation remains unsatisfactory, and alternative approaches are needed to prevent the occurrence of fractures and the associated morbidity.

Denosumab is a fully human monoclonal antibody against Receptor Activator of Nuclear Factor κB Ligand (RANKL), which was developed for treatment of osteoporosis and prevention of fractures (15). By inhibiting the development and the activity of osteoclasts, denosumab decreases bone resorption and increases bone density. Compared with bisphosphonates, denosumab proved to have superior efficacy in improving BMD and preventing fractures in post-menopausal women with osteoporosis (16, 17). Since current treatment options for post-transplant bone loss are limited, we studied the efficacy and safety of RANKL inhibition with denosumab in *de novo* kidney transplant recipients.

Materials and Methods

This study was a 1-year prospective single-center, randomized, parallel-group, open-label clinical trial in *de novo* kidney transplant recipients, performed in an academic setting at the University Hospital Zürich (Switzerland). The sponsor of the study was the University Hospital Zürich and the University of Zürich. The study conformed to the principles of the

Declaration of Helsinki and the Declaration of Istanbul as outlined in the "Declaration of Istanbul on Organ Trafficking and Transplant Tourism." The local Ethics Committee approved the study protocol and acknowledged the statistical analysis plan (IRB approval number 2011-0032). All patients provided written informed consent before participating. Certified external monitors evaluated the course and the quality of the study at regular intervals. This trial is registered with ClinicalTrials.gov, NCT01377467.

Participants and Study Design

Adult male and female end-stage renal disease patients were eligible for inclusion if transplanted up to 28 days ago with a kidney allograft and being treated with standard triple immunosuppression including a calcineurin antagonist, mycophenolate and corticosteroids. Key exclusion criteria were poor or unstable graft function (creatinine >200 µmol/l), severe osteoporosis (T-score below -4.0), severe hyper- or hypoparathyroidism (iPTH >800 or <10 ng/l), and hypo- or hypercalcaemia (total calcium <1.8 or >2.7 mmol/l). All patients were prescribed daily supplements of calcium (1000 mg), and vitamin D (800 IU or more).

After baseline assessment, male and female subjects were randomly assigned (1:1) to receive subcutaneous injections of 60 mg denosumab at baseline and after 6 months, or no treatment. The randomization list was generated by the hospital pharmacist with a computer algorithm prior to study initiation, using a permuted blocks design with block sizes of 4 and 6. Allocation concealment was ensured by the use of sequentially numbered, opaque, sealed envelopes. Study physicians and nurses and participants were aware of the allocated group. The persons that performed dual-energy x-ray absorptiometry (DXA), high-resolution peripheral quantitative computer tomography (HR-pQCT) and biomarker measurements were masked to allocation.

Study Assessments

Study visits were performed at baseline and months 0.5, 1, 2, 3, 6 and 12, when clinical and routine laboratory values were captured in a secured web-based case report form (secuTrial®, interActive Systems). Graft function was assessed by calculating the eGFR with the creatinine-based CKD-EPI formula.

Levels of parathyroid hormone (PTH), 25-(OH)-vitamin D, 1,25-$(OH)_2$-vitamin D, procollagen type I N-terminal propeptide (P1NP), bone-specific alkaline phosphatase (BSAP), and β-isomer of the C-terminal telopeptide of type I collagen (β-CTX) were assessed in serum samples at baseline and months 3, 6, and 12. Urine deoxypyridinoline and creatinine were measured at baseline, and months 1, 3, 6, and 12.

Measurements of areal BMD (aBMD) were performed at the lumbar spine and proximal femur by DXA at baseline and at 6 and 12 months in all patients, using a bone densitometer of the QDR series in array mode (Hologic Discovery®). Quality controls using the Hologic spine phantom were performed daily before the first patient scan. Least significant changes for a 95% level of confidence at the lumbar spine (L1 – L4) were as follows: root mean square standard deviation 0.029 g/cm^2, coefficient of variation 0.030, percent coefficient of variation 3.04%.

A subset of 24 consecutively randomized patients underwent volumetric BMD (vBMD) examination by HR-pQCT (XtremeCT®, Scano Medical, Switzerland) at baseline and after 12 months to assess changes in cortical and trabecular bone mineralization and cortical thickness at the distal tibia and radius.

Outcomes

The primary endpoint was the percentage change in baseline aBMD at the total lumbar spine at 12 months. Secondary endpoints included changes in aBMD at total hip and femoral neck, and changes in biomarkers of bone turnover. Adverse events (AE) were reported

spontaneously and in response to nondirected questioning at each study visit, and were coded and graded according to the Common Terminology Criteria for Adverse Events (CTCAE). Hypo- and hypercalcemia were defined as an albumin-adjusted calcium level of <1.9 and >2.6 mmol/l, respectively.

Sample Size Estimation

We calculated that a sample size of 43 patients per group would provide a statistical power of 86% to detect a 4% difference in the percentage change of aBMD at the total lumbar spine at 12 months, using a two-sided t-test with an α-level of 0.05 and assuming a mean ± SD change of 4 ± 6% in the denosumab group and 0 ± 6% in the control group. To account for a dropout rate of 5%, it was planned to randomize a total of 90 patients.

Statistical Analysis

Analyses of efficacy and safety were based on the intention-to-treat principle. For aBMD data, missing values were replaced with a last-observation-carried-forward approach; for all other efficacy endpoints, an available case analysis was performed. The primary efficacy endpoint, the percentage change in aBMD at the total lumbar spine at 12 months, was analyzed using a linear model with treatment as fixed effect and baseline aBMD as covariate.

The exact Wilcoxon rank sum test was used to compare the percentage changes between baseline and 12 months for HR-pQCT parameters. Changes of bone mineral and metabolism biomarkers and changes of renal function were analyzed using a general linear model for repeated measures.

For the most frequent AE, the number of affected patients was compared between the two treatment groups using the chi-square test. The number of bacterial, viral or fungal infections was compared between the two treatment groups with the exact Wilcoxon rank sum test.

Statistical analyses were done using IBM SPSS Statistics Version 20.

Results

Baseline Clinical Characteristics

The study population included 90 kidney transplant recipients with a functioning graft. Patients were recruited from June 20, 2011, to May 2, 2014. Patients were randomized after 15.7 ± 6.4 days after transplantation to denosumab treatment (*n*=46) at a dose of 60 mg subcutaneously at baseline and at 6 months, or no treatment (*n*=44). Two patients did not receive denosumab and another two patients received only the baseline dose (Figure 1).

Baseline characteristics were balanced between the two groups, except for more men and a correspondingly higher baseline aBMD in the denosumab group (Table 1). All patients received triple immunosuppression therapy with a calcineurin inhibitor (70% tacrolimus, 30% cyclosporine), mycophenolate and corticosteroids. The cumulative amount of corticosteroids over the 12-months study period was similar, amounting to 3.760 ± 1.445 g in the denosumab and 3.974 ± 1.727 g in the control group.

At baseline 45.6% of all patients had osteopenia and 10.0% had osteoporosis. All patients were prescribed vitamin D and calcium throughout the study, receiving a daily dose of 1526 ± 946 vs 1400 ± 1295 IU vitamin D and 756 ± 609 vs 619 ± 475 mg calcium at month 12 in the denosumab and control group, respectively. During the 12-month study period two patients in the denosumab group were initiated on cinacalcet and one on calcitriol. None of the patients received paricalcitol, bisphosphonates or other drugs with specific effects on bone metabolism.

Table 2 shows the parameters of renal function and mineral metabolism at baseline, 3, 6 and 12 months. The eGFR was similar and stable in the two treatment arms. Serum calcium and phosphate increased and were similar, whereas PTH decreased more rapidly in the control group. The serum levels of 25-(OH)-vitamin D and 1,25-$(OH)_2$-vitamin D progressively increased in both groups to similar levels at 12 month.

Change in areal Bone Mineral Density

Denosumab treatment was associated with increased aBMD at all measured sites (Figure 2, Table 3 and Table 4). Denosumab increased aBMD over that in the control group at 12 months at the total lumbar spine by 5.1% (P<0.0001) and at the total hip by 1.9% (P=0.035). The effect of denosumab was significant at 6 months at the total lumbar spine (P<0.0001) and total hip (P=0.009), but not quite significant at the femoral neck (P=0.064). Denosumab also significantly increased the T-scores at the total lumbar spine at 6 and 12 months, and at the total hip at 6 months (Table 4). Subgroup analysis revealed that the effect of denosumab on lumbar spine aBMD at 12 months was consistent, with slightly better effects in patients that were younger and of male sex, and in patients having lower T-scores, higher eGFR, and lower PTH levels (Figure 2D).

In a subgroup of 24 randomized patients, we analyzed vBMD at the distal tibia and radius at baseline and 12 months by HR-pQCT (Table 5). Treatment with denosumab was associated with a significant increase of the average vBMD and the cortical thickness at both sites. The cortical vBMD increased significantly at the tibia, whereas the changes of the trabecular vBMD were not significant.

Change in Biomarkers of Bone Turnover

Serum and urine levels of biochemical markers of bone resorption and bone formation decreased significantly in the denosumab group when compared to the control group (Figure 3). The between-subjects effect was significant for all biomarkers (P<0.0001 for β-CTX, P1NP, and BSAP; P=0.004 for deoxypyridinoline:creatinine ratio). Moreover, the time*treatment interaction was significant for β-CTX (P=0.002) and P1NP (P=0.012), indicating that the course over time for β-CTX and P1NP was also different in the two study arms. Thus, bone turnover decreased significantly in the denosumab group, whereas it remained unchanged in the control group.

Safety

All 90 patients had complete follow-up during the 12 month study period. There were 349 adverse events (AE) in the denosumab (7.6 per patient) and 273 in the control group (6.2 per patient), of which 60 (17%) and 52 (19%), respectively, were serious adverse events (SAE) (Table 6). There were no unexpected AE or SAE and no deaths. Overall, AE were of mild or moderate degree and of similar severity in both groups. The most common AE were urinary tract infection (cystitis), diarrhea, CMV viremia, cough, leg pain, flu-like disease, and polyoma (BK) viremia, which occurred in more than 20% of the patients. Urinary tract infections (P=0.008) and diarrhea (P=0.048) occurred in more patients in the denosumab than in the control group. In patients with urinary tract infection the spectrum of urine bacteria was similar in the denosumab and control group, but *E. coli* (20/45 vs 9/29 positive cultures) and *Enterococcus faecalis* (8/45 vs 1/29 positive cultures) occurred more frequently in the denosumab than in the control group. A total of 146 vs 99 infections were counted in the denosumab and the control group, respectively. Patients in the denosumab group thus experienced a higher number of infections than patients in the control group (P=0.044). Transplant-related AE occurred with similar frequency in both groups, including CMV viremia, BK viremia, lymphocele, transplant pyelonephritis, and episodes of acute rejection. Renal function remained stable with no difference in serum creatinine, eGFR and proteinuria at 12 months. There was one case of graft failure due to BK virus nephropathy and rejection in the denosumab group. There was one case of a traumatic rib fracture occurring in a patient with osteoporosis in the control group. Episodes of asymptomatic and transient hypocalcemia (<1.9 mmol/l) occurred more frequently in the denosumab group (12 vs 1), whereas episodes of hypercalcemia (>2.6 mmol/l) were less frequent (37 vs 55).

Discussion

In *de novo* kidney transplant recipients on corticosteroid-containing immunosuppression, the administration of denosumab significantly increased aBMD at vertebral and non-vertebral sites. Denosumab also improved cortical vBMD and cortical thickness at the distal tibia and radius. As such denosumab differs from other drugs such as bisphosphonates, which so far failed to demonstrate any positive effect on cortical bone. Furthermore denosumab significantly decreased the levels of blood and urine biomarkers of bone turnover. The beneficial effects of denosumab appeared robust and were seen in all subgroups.

Patients with end-stage renal disease presenting for kidney transplantation often have a reduced bone mass and an increased risk for fracture, particularly if they had previous corticosteroid treatment for glomerulonephritis or if they had long-standing secondary hyperparathyroidism (1, 2, 4). Kidney transplantation is associated with a further loss of bone mass of up to 10% in the first year after grafting (14, 18), although more recent studies - and our study as well - documented less bone loss, possibly because of a lower use of corticosteroids and an increased use of calcium and vitamin D supplementation (1, 2, 12, 19). Patients with heightened risk for rejection, however, may not qualify for corticosteroid-free immunosuppression and need alternative approaches to preserve their bone mass.

Follow-up studies of transplant recipients have documented that one in five patients develops a fracture within five years after transplantation (20, 21). Several therapeutic schemes were shown to prevent the loss of bone mass and to possibly improve the fracture risk in transplant recipients, including supplementation with calcium and/or vitamin D and treatment with bisphosphonates (12, 22). While calcium and vitamin D supplementation improve the first year loss of bone mass in renal transplantation, the addition of a bisphosphonate does not appear to increase bone mass at all skeletal sites (6, 10). Whereas calcium and vitamin D are therefore often given after transplantation, bisphosphonates are not frequently prescribed, particularly because of limited efficacy and the well-known side

effects. Paricalcitol and cinacalcet improve persistent hyperparathyroidism after transplantation, but these drugs do also not appear to have a major effect on BMD (23-25). Thus, despite progress, the management of bone loss after transplantation has remained unsatisfactory.

This study shows that denosumab effectively increased BMD in kidney transplant recipients, comparable to its effect in postmenopausal women with osteoporosis and other patient groups (16). This suggests that denosumab's interference with the RANKL system is effective in diverse situations of bone loss. In the setting of renal transplantation this does not only include effective antagonism of corticosteroid-induced loss of bone mass, but also counteracting high-turnover bone disease from persistent hyperparathyroidism. Therefore this study extends the spectrum of patients benefiting from RANKL antagonism to kidney transplant recipients and potentially to recipients of other organ transplants at high risk of osteoporosis and fracture.

Only limited data are available regarding fracture prevention in kidney transplant recipients. In retrospective analyses, corticosteroid minimization was associated with preservation of bone mass and a reduction of the fracture risk (19). Whether bisphosphonates decrease the fracture risk is debated (11, 12). Given that denosumab reduces the occurrence of fractures in osteoporotic patients with chronic renal failure we would predict that it should also be effective in preventing fractures in kidney transplant recipients (26), but this would need to be examined in appropriately powered studies.

Our study has limitations, which includes a slight imbalance in patient characteristics between the two groups that is likely due to a relatively low sample size. However, subgroup analysis revealed that the effect of denosumab on aBMD was consistent. From a conceptual point of view the treatment duration and the follow-up were short, wherefore the study could not address the risk of fracture. This would obviously require a much larger patient number and prolonged treatment time, but this was not the scope of the present study. Also, it might

be argued that treatment with denosumab could increase the risk for low-turnover bone disease, as seen in patients with renal failure after extended treatment with active vitamin D, calcium-containing phosphate binders or bisphosphonates (27, 28), and also in patients with a renal transplant (29). Since we did not perform bone biopsies we could not firmly assess whether denosumab promoted low-turnover bone disease. However, the profiles and the levels of the biomarkers of bone resorption (β-CTX and urine deoxypyridinoline) and bone formation (P1NP and BSAP) showed that bone turnover was not entirely suppressed with denosumab treatment, but rather improved towards normal levels. Persistent hyperparathyroidism may possibly counteract the development of low-turnover bone disease in denosumab-treated kidney transplant recipients. From a general point of view it may be argued that an increase in BMD in renal transplant patients as measured by DXA or HR-pQCT may not necessarily translate into a subsequent reduction of fracture risk. On the other hand denosumab-induced increases in BMD have been shown to correlate very well with a reduced fracture risk in various patient populations, including patients with renal failure (26).

Urinary tract infections are common in kidney transplant recipients (30). Treatment with denosumab was associated with an increase of the incidence of these infections in our study. Patients mainly developed cystitis which responded rapidly to antibiotic treatment; the incidence of pyelonephritis and urosepsis was not increased. A slightly higher number of infections of the renal and urinary system has previously been noticed in denosumab-treated postmenopausal women (31). The RANKL system may play a role in specific and non-specific immune responses, and the inhibition of RANKL by denosumab might influence the resistance to microbial organisms, however at this point the mechanisms which could explain the increased incidence of urinary tract infections remain unclear (32, 33).

In conclusion, denosumab significantly increased BMD at all measured skeletal sites and reduced biomarkers of bone turnover in kidney transplant recipients. Except for a higher number of urinary tract infections and asymptomatic episodes of hypocalcemia, denosumab

was safe. Denosumab treatment may therefore be useful to improve bone health in the first year after kidney transplantation.

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Disclosure

The authors of this manuscript have conflicts of interest to disclose as described by the American Journal of Transplantation. RPW reports personal fees from Amgen, outside the submitted work. NG reports personal fees from Boehringer Ingelheim GmbH, medac GmbH Bayer, outside the submitted work. A part of the study drug was obtained free of charge by Amgen Switzerland AG. The other authors have no conflicts of interest to disclose.

Figure Legends

Figure 1: Study flow chart

Figure 2: Within-group change in aBMD (%) from baseline to 6 months and to 12 months by DXA at total lumbar spine (A), total hip (B) and femoral neck (C). Data are least-squares means adjusted for baseline aBMD, error bars indicate 95% CI. *** P<0.0001, ** P <0.01, * P <0.05. Forest plot (D), showing least-squares means and 95% CI for primary endpoint (% change in aBMD at the total lumbar spine at 12 months), stratified for age, sex, baseline T-score, baseline GFR and baseline PTH. Numbers in brackets show number of patients. aBMD, areal bone mineral density; DXA, dual-energy x-ray absorptiometry; GFR, glomerular filtration rate.

Figure 3: Course of serum β-CTX (A), serum P1NP (B), urine deoxypyridinoline to creatinine ratio (C) and serum BSAP (D) from baseline to month 12 in the control and denosumab group. Data are least-squares means, and error bars indicate 95% CI. Analyses include

patients with valid data at all timepoints. Thus for β-CTX 42 and 42 patients, for P1NP 41 and 42 patients, for urine deoxypyridinoline:creatinine ratio 25 and 21 patients, and for BSAP 41 and 42 patients from the control and denosumab group, respectively, were included. β-CTX, C-terminal telopeptide of type I collagen; BSAP, bone-specific alkaline phosphatase; P1NP, procollagen type I N-terminal propeptide.

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Table 1: Baseline characteristics of patients

	Control (*n*=44)	**Denosumab (*n*=46)**
Age (years)	49.0 ± 12.9	50.0 ± 14.0
Men	22 (50.0%)	35 (76.1%)
White ethnicity	42 (95.5%)	44 (95.7%)
Body-mass index (kg/m^2)	25.5 ± 5.3	25.8 ± 4.6
Pre-transplant dialysis mode		
Hemodialysis	31 (70.5%)	26 (56.5%)
Peritoneal dialysis	9 (20.5%)	7 (15.2%)
Pre-emptive transplantation	4 (9.1%)	13 (28.3%)
Repeat transplantation	7 (15.9%)	7 (15.2%)
Transplant from deceased donor	26 (59.1%)	18 (39.1%)
Number of HLA mismatches	3.8 ± 1.3	3.5 ± 1.3
Panel reactive antibody titer ≥20%	0 (0.0%)	0 (0.0%)
Cause of end-stage renal disease		
Chronic glomerulonephritis	11 (25.0%)	19 (41.3%)
Diabetic nephropathy	3 (6.8%)	4 (8.7%)
Hypertensive/vascular nephropathy	6 (13.6%)	2 (4.3%)
Polycystic kidney disease	8 (18.2%)	12 (26.1%)
Other hereditary	4 (9.1%)	1 (2.2%)
Other	12 (27.3%)	8 (17.4%)
Immunosuppression		
Induction therapy †	44 (100.0%)	45 (97.8%)
Tacrolimus	33 (75.0%)	30 (65.2%)
Cyclosporine	11 (25.0%)	16 (34.8%)
Mycophenolate	44 (100.0%)	46 (100.0%)

Corticosteroids	44 (100.0%)	46 (100.0%)
Areal bone mineral density (aBMD) and T-scores		
Total lumbar spine aBMD (g/cm^2)	0.934 ± 0.129	1.002 ± 0.139
Total lumbar spine T-score	-1.27 ± 1.15	-0.67 ± 1.25
Total hip aBMD (g/cm^2)	0.847 ± 0.092	0.925 ± 0.132
Total hip T-score	-1.05 ± 0.65	-0.59 ± 0.97
Number of osteopenic patients	25 (56.8%)	16 (34.8%)
Number of osteoporotic patients	6 (13.6%)	3 (6.5%)

Data are mean ± SD or number (%). † Patients received basiliximab (68.9%) or anti-thymocyte globulin (30.0%). aBMD, areal bone mineral density.

Table 2: Parameters of renal function and mineral metabolism

Parameter	**Visit**	**Control**	**Denosumab**	***P*-values**	
				within-subjects effect	*between-subjects effect*
eGFR (ml/min/1.73 m^2) *n=44 (control), n=44 (denosumab)*	Baseline	54.2 ± 16.2	53.5 ± 15.4	0.704	0.820
	3 months	52.9 ± 15.1	56.0 ± 17.2		
	6 months	55.0 ± 15.3	54.2 ± 17.9		
	12 months	55.2 ± 20.7	56.4 ± 17.4		
Calcium (mmol/l) *n=42 (control), n=39 (denosumab)*	Baseline	2.33 ± 0.22	2.31 ± 0.16	<0.001	0.198
	3 months	2.47 ± 0.19	2.40 ± 0.21		
	6 months	2.47 ± 0.17	2.46 ± 0.15		
	12 months	2.52 ± 0.16	2.47 ± 0.15		
Phosphate (mmol/l) *n=41 (control), n=40 (denosumab)*	Baseline	0.57 ± 0.18	0.58 ± 0.23	<0.001	0.890
	3 months	0.76 ± 0.16	0.78 ± 0.33		
	6 months	0.83 ± 0.19	0.82 ± 0.28		
	12 months	0.89 ± 0.18	0.85 ± 0.37		
25-(OH)-vitamin D (µg/l) *n=40 (control), n=41 (denosumab)*	Baseline	18.1 ± 9.0	17.4 ± 8.3	<0.001	0.578
	3 months	22.9 ± 7.1	21.6 ± 5.9		
	6 months	26.7 ± 11.6	24.4 ± 7.1		
	12 months	28.4 ± 8.9	28.5 ± 8.2		
1,25-$(OH)_2$-vitamin D (ng/l) *n=34 (control), n=36 (denosumab)*	Baseline	34.2 ± 24.6	29.6 ± 21.0	<0.001	0.607
	3 months	55.3 ± 14.5	60.5 ± 29.7		
	6 months	58.1 ± 22.5	54.2 ± 21.4		
	12 months	51.5 ± 20.0	47.2 ± 23.7		
PTH (ng/l) *n=40 (control), n=40 (denosumab)*	Baseline	147.3 ± 141.6	163.1 ± 157.9	<0.001	0.114
	3 months	111.6 ± 85.4	173.6 ± 175.5		
	6 months	99.4 ± 64.7	157.0 ± 167.4		
	12 months	100.7 ± 67.3	106.7 ± 69.7		

Course of renal function (eGFR as determined by the CKD-EPI formula) and blood parameters of mineral metabolism at baseline, 3, 6 and 12 months. Data are mean ± SD. PTH was the only parameter with a significant time*treatment interactions (*P*=0.047). Adjustment for multiplicity was not performed. eGFR, estimated glomerular filtration rate; PTH, parathyroid hormone.

Table 3: Between-group differences in aBMD (%) and T-scores by DXA

Change from baseline	**B**	**SE**	**95% CI**	***P* Values**
Lumbar spine aBMD				
Month 6	4.6	0.8	3.0 – 6.2	**<0.0001**
Month 12	5.1	1.0	3.1 – 7.0	**<0.0001**
Lumbar spine T-score				
Month 6	0.39	0.07	0.24 – 0.53	**<0.0001**
Month 12	0.42	0.08	0.26 – 0.59	**<0.0001**
Total hip aBMD				
Month 6	1.8	0.7	0.4 – 3.1	**0.009**
Month 12	1.9	0.9	0.1 – 3.7	**0.035**
Total hip T-score				
Month 6	0.10	0.04	0.01 – 0.18	**0.028**
Month 12	0.10	0.05	-0.01 – 0.20	0.075
Femoral neck aBMD				
Month 6	2.0	1.1	-0.1 – 4.2	0.064
Month 12	1.1	1.2	-1.3 – 3.4	0.380
Femoral neck T-score				
Month 6	0.10	0.07	-0.04 – 0.23	0.152
Month 12	0.02	0.07	-0.12 – 0.17	0.738

Between-group differences (denosumab – control) in aBMD (percentage) and T-scores from baseline to 6 and to 12 months, adjusted for baseline aBMD and T-score, respectively. Data are least-squares means and 95% CI. B, regression coefficients; SE, standard errors of the regression coefficients. N=44 (control) and n=46 (denosumab) for aBMD; n=43 (control) and n=43 (denosumab) for T-scores. Adjustment for multiplicity was not performed. aBMD, areal bone mineral density; DXA, dual-energy x-ray absorptiometry.

Table 4: Within-group changes in aBMD (%) and T-scores by DXA

Parameter			
DXA	**Control (*n*=44)**	**Denosumab (*n*=46)**	***P* Values**
Total lumbar spine aBMD (g/cm^2)			
Change from baseline to 6 months	-1.6 (-2.7 – -0.5)	3.0 (1.9 – 4.1)	**<0.0001**
Change from baseline to 12 months	-0.5 (-1.8 – 0.9)	4.6 (3.3 – 5.9)	**<0.0001**
Total lumbar spine T-score			
Change from baseline to 6 months	-0.13 (-0.23 – -0.03)	0.25 (0.16 – 0.35)	**<0.0001**
Change from baseline to 12 months	-0.02 (-0.14 – 0.10)	0.40 (0.28 – 0.52)	**<0.0001**
Total hip aBMD (g/cm^2)			
Change from baseline to 6 months	-0.3 (-1.2 – 0.6)	1.4 (0.5 – 2.3)	**0.009**
Change from baseline to 12 months	0.4 (-0.8 – 1.7)	2.3 (1.1 – 3.5)	**0.035**
Total hip T-score			
Change from baseline to 6 months	-0.01 (-0.07 – 0.05)	0.09 (0.03 – 0.15)	**0.028**
Change from baseline to 12 months	0.04 (-0.03 – 0.12)	0.14 (0.07 – 0.22)	0.075
Femoral neck spine aBMD (g/cm^2)			
Change from baseline to 6 months	-0.9 (-2.4 – 0.6)	1.1 (-0.3 – 2.6)	0.064
Change from baseline to 12 months	0.4 (-1.2 – 2.1)	1.5 (-0.1 – 3.1)	0.380
Femoral neck T-score			
Change from baseline to 6 months	-0.05 (-0.14 – 0.05)	0.05 (-0.04 – 0.14)	0.152
Change from baseline to 12 months	0.03 (-0.07 – 0.13)	0.05 (-0.05 – 0.15)	0.738

Within-group changes in aBMD (percentage) and T-scores from baseline to 6 and to 12 months. Data are least-squares means and 95% CI. *P* values indicate significance for between-group differences. Adjustment for multiplicity was not performed. aBMD, areal bone mineral density; DXA, dual-energy x-ray absorptiometry.

Table 5: Effect of denosumab on changes in vBMD and cortical thickness by HR-pQCT

HR-pQCT	Control (*n*=14)	Denosumab (*n*=10)	*P* Values
Distal tibia			
Average vBMD	-0.3 (-3.3 – 2.7)	2.2 (0.7 – 3.2)	**0.019**
Cortical vBMD	-0.5 (-2.0 – 0.1)	0.1 (-0.5 – 1.4)	**0.042**
Trabecular vBMD	1.1 (-2.5 – 4.1)	1.8 (1.3 – 4.2)	0.371
Cortical thickness	-0.9 (-5.9 – 1.6)	2.8 (1.4 – 8.0)	**0.005**
Distal radius			
Average vBMD	-1.6 (-5.2 – 0.7)	1.3 (-1.9 – 2.2)	**0.031**
Cortical vBMD	-0.9 (-2.7 – 0.4)	-0.1 (-1.8 – 1.3)	0.212
Trabecular vBMD	0.2 (-9.4 – 3.1)	2.4 (1.1 – 4.1)	0.108
Cortical thickness	-3.6 (-7.7 – -1.3)	0.9 (-6.0 – 2.0)	**0.048**

Percentage change in vBMD (mg HA/cm^3) and cortical thickness (mm). Data show median and 95% CI based on quantiles of the binomial distribution. Adjustment for multiplicity was not performed. vBMD, volumetric bone mineral density.

Table 6: Adverse events in the safety analysis population

Adverse events	Control group (*n*=44)		Denosumab group (*n*=46)	
	No. events (%)	No. patients (%)	No. events (%)	No. patients (%)
Urinary tract infection**	25 (9.2%)	11 (25.0%)	51 (14.6%)	24 (52.2%)
Transplant pyelonephritis	5 (1.8%)	5 (11.4%)	3 (0.9%)	3 (6.5%)
Diarrhea*	14 (5.1%)	13 (29.5%)	32 (9.2%)	23 (50.0%)
CMV viremia	24 (8.8%)	18 (40.9%)	26 (7.4%)	20 (43.5%)
Cough	19 (7.0%)	12 (27.3%)	9 (2.6%)	7 (15.2%)
Leg pain	12 (4.4%)	9 (20.5%)	14 (4.0%)	9 (19.6%)
Flu-like disease	14 (5.1%)	12 (27.3%)	12 (3.4%)	11 (23.9%)
Polyoma (BK) viremia	11 (4.0%)	11 (25.0%)	12 (3.4%)	12 (26.1%)
Abdominal pain	10 (3.7%)	8 (18.2%)	10 (2.9%)	9 (19.6%)
Leg edema	5 (1.8%)	5 (11.4%)	10 (2.9%)	9 (19.6%)
Lymphocele	9 (3.3%)	8 (18.2%)	7 (2.0%)	7 (15.2%)
Herpes labialis	3 (1.1%)	3 (6.8%)	8 (2.3%)	5 (10.9%)
Acute rejection	4 (1.5%)	3 (6.8%)	5 (1.4%)	5 (10.9%)
Fracture	1 (0.4%)	1 (2.3%)	0 (0.0%)	0 (0.0%)
Loss of graft function	0 (0.0%)	0 (0.0%)	1 (0.3%)	1 (2.2%)
Other	117 (42.9%)	36 (81.8%)	149 (42.7%)	42 (91.3%)
Total	273 (100.0%)	44 (100.0%)	349 (100.0%)	45 (97.8%)

* P=0.048; ** P=0.008